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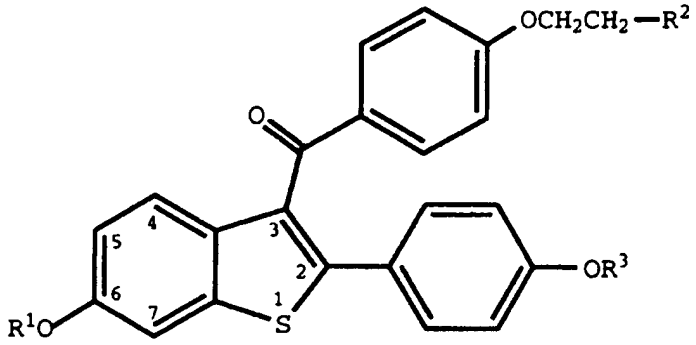
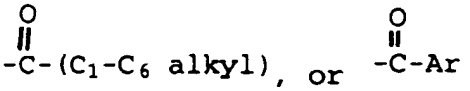
(54) Inhibition of autoimmune diseases.

(57) A method of inhibiting autoimmune diseases comprising administering to a human in need thereof an effective amount of a compound having the formula



(I)

wherein R^1 and R^3 are independently hydrogen, $-CH_3$,



wherein Ar is optionally substituted phenyl ;

R^2 is select d from the group consisting of pyrrolidine, hexamethyl neamino, and piperidino ; or a pharmaceutically acceptable salt of solvate thereof.

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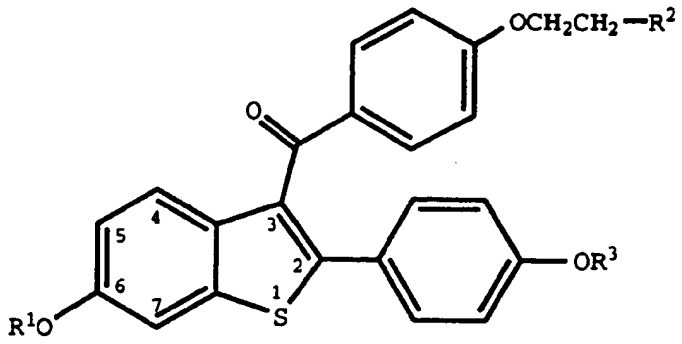
Autoimmune diseases involve aberrant regulation of cellular and humoral mediated immunity and are frequently associated with abnormal or enhanced T cell, B cell and macrophage effector functions directed towards self antigens. The activation of these cellular components towards self antigens is believed related to the break in feedback mechanisms associated with self tolerance. Autoimmune diseases encompass a whole spectrum of clinical entities and despite the differences in the target organ have many similarities. These include their preponderance in females of child bearing age with a female to male ratio varying from 50:1 in Hashimoto's throiditis to 10:1 in Systemic lupus erythematosus to 2:1 in Myasthenia gravis (Ahmed *et al., Am J. Path., 121*:531 (1985)). In addition, these diseases are all characterized by their chronicity, the tendency of clinical remission and "flare ups" for poorly understood reasons, and the involvement of other organs. While the presence of autoantibodies, inappropriate expression of class II antigens, macrophage activation and T cell infiltration to the target organ have been described in essentially all of the autoimmune diseases, neither the triggering mechanisms which result in disease activation nor disease progression are well understood. Accordingly, therapy for these diseases is largely unsatisfactory and involves the use of gold salts, methotrexate, antimalarials, glucocorticoids (methylprednisolone), and other immunosuppressives as well as plasmaphoresis and attempts at inducing tolerance. Treatment of autoimmune diseases has not improved significantly over the past decade and primarily is associated with the use of nonsteroidal and steroidal anti-inflammatory agents to treat the symptoms of the disease. Clearly while suppression of the specific immune response directed against the host is necessary, generalized immunosuppression as with glucocorticoids has major liabilities in terms of side effect profile and the propensity of the immunosuppressed patient to be at greater risk for other infectious and non-infectious diseases.

Estrogen appears to be involved with autoimmune diseases although its role in disease progression or regression is complex and dependent on the nature of the autoimmune disease. Estrogen for example appears to have an ameliorating effect on rheumatoid arthritis while having an exacerbating effect on systemic lupus (Chander & Spector; *Ann. Rheum. Dis. 50*:139). Estrogen has been demonstrated to have a suppressive role on T cell function and yet an immunostimulatory effect on B cells. Therefore, estrogen-like compounds should prove beneficial in diseases associated with activated T cells including rheumatoid arthritis, multiple sclerosis, Guillan Barre syndrome and Hashimoto's thyroiditis through inhibition of T cell function (Holmdahl, *J. Autoimmun. 2*:651 (1989).

In addition to the suppressive effects of estrogen on T cells, estrogen may have additional protective roles. Marui et al., (*J. Clin. Invest. 92*:1866 (1993)) have recently reported that antioxidants suppress endothelial expression of VCAM-1. VCAM-1 is the ligand for VLA-4, the T cell and macrophage integrin associated with trafficking of these cells out of the vasculature and into the perivascular space and target organs. As estrogen is an antioxidant, it would be anticipated that estrogen and related analogs will inhibit VLA-4 dependent trafficking of cells and thus hinder the immune cascade associated with autoimmune mediated disease.

Estrogen plays a detrimental role in other autoimmune diseases including systemic lupus and glomerulonephritis, diseases associated with immune complexes. While the mechanism(s) responsible for estrogen mediated disease progression are not known, the ability of estrogen to increase Fc mediated phagocytosis (Friedman *et al., J. Clin. Invest.* 75:162 (1985), and class II antigen expression and IL-1 production by macrophages from estrogen treated rodents (Flynn, *Life Sci., 38*:2455 (1986) has been reported. Enhancement of these macrophage mediated effector functions would be expected to contribute towards the immune cascade associated with self destruction.

This invention provides methods for inhibiting autoimmune diseases comprising administering to a human in need thereof an effective amount of a compound of formula I



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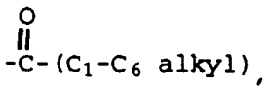

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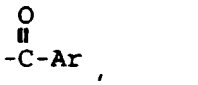


wherein Ar is optionally substituted phenyl;

R^2 is selected from the group consisting of pyrrolidino, hexamethyleneimino, and piperidino; and pharmaceutically acceptable salts and solvates thereof.

The current invention concerns the discovery that a select group of 2-phenyl-3-aroylbenzothiophenes (benzothiophenes), those of formula I, are useful for inhibiting autoimmune diseases and their symptoms. It is believed the benzothiophenes disclosed are active against autoimmune diseases by inhibition of T cell function, inhibition of class II antigen expression thereby inhibiting macrophage mediated antigen presentation, and/or inhibition of release of cytokines including IL-1, TNF, and other inflammatory mediators. The therapeutic and prophylactic treatments provided by this invention are practiced by administering to a human in need thereof a dose of a compound of formula I or a pharmaceutically acceptable salt or solvate thereof, that is effective to inhibit autoimmune disease or its symptoms.

The term "inhibit" includes its generally accepted meaning which includes prohibiting, preventing, restraining, and slowing, stopping or reversing progression, severity or a resultant symptom. As such, the present method includes both medical therapeutic and/or prophylactic administration, as appropriate.

An autoimmune disease involves aberrant regulation of cellular and humoral mediated immunity and is frequently associated with abnormal or enhanced T cell, B cell, or macrophage effector functions directed toward self-antigen. Examples of autoimmune diseases includes systemic lupus erythrematosas, Hashimoto's thyroiditis, myasthenia gravis, rheumatoid arthritis, multiple sclerosis, Guillan Barre syndrome, and glomerulonephritis.

Raloxifene is a preferred compound of this invention and it is the hydrochloride salt of a compound of formula 1 wherein R^1 and R^3 are hydrogen and R^2 is 1-piperidinyl.

Generally, at least one compound of formula I is formulated with common excipients, diluents or carriers, and compressed into tablets, or formulated as elixirs or solutions for convenient oral administration, or administered by the intramuscular or intravenous rout s. Th compounds can be administer d transd rmally, and may b formulated as sustain d releas dosag forms and the lik .

Th compounds used in th m thods of the current inv ntion can b made according to established procedures, such as those detailed in U.S. Patent Nos. 4,133,814, 4,418,068, and 4,380,635 all of which are incorporated by r ference herein. In g n ral, the process starts with a benzo[b]thioph ne having a 6-hydroxyl

group and a 2-(4-hydroxyph nyl) group. Th starting compound is prot cted, acylated, and d protected to form the formula I compounds. Examples of the pr paration of such compounds are provided in the U.S. patents discussed abov . The t rm "optionally substituted phenyl" includes phenyl and phenyl substituted once or twice with C_1-C_6 alkyl, C_1-C_4 alkoxy, hydroxy, nitro, chloro, fluoro, or tri(chloro or fluoro)m thyl.

The compounds used in the methods of this invention form pharmaceutically acceptable acid and base addition salts with a wide variety of organic and inorganic acids and bases and include the physiologically acceptable salts which are often used in pharmaceutical chemistry. Such salts are also part of this invention. Typical inorganic acids used to form such salts include hydrochloric, hydrobromic, hydroiodic, nitric, sulfuric, phosphoric, hypophosphoric and the like. Salts derived from organic acids, such as aliphatic mono and dicarboxylic acids, phenyl substituted alkanoic acids, hydroxyalkanoic and hydroxyalkandioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, may also be used. Such pharmaceutically acceptable salts thus include acetate, phenylacetate, trifluoroacetate, acrylate, ascorbate, benzoate, chlorobenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, methylbenzoate, o-acetoxybenzoate, naphthalene-2-benzoate, bromide, isobutyrate, phenylbutyrate, β-hydroxybutyrate, butyne-1,4-dioate, hexyne-1,4-dioate, caprate, caprylate, chloride, cinnamate, citrate, formate, fumarate, glycollate, heptanoate, hippurate, lactate, malate, maleate, hydroxymaleate, malonate, mandelate, mesylate, nicotinate, isonicotinate, nitrate, oxalate, phthalate, teraphthalate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, propiolate, propionate, phenylpropionate, salicylate, sebacate, succinate, suberate, sulfate, bisulfate, pyrosulfate, sulfite, bisulfite, sulfonate, benzene-sulfonate, p-bromophenylsulfonate, chlorobenzenesulfonate, ethanesulfonate, 2-hydroxyethanesulfonate, methanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, p-toluenesulfonate, xylenesulfonate, tartarate, and the like. A preferred salt is the hydrochloride salt.

The pharmaceutically acceptable acid addition salts are typically formed by reacting a compound of formula I with an equimolar or excess amount of acid. The reactants are generally combined in a mutual solvent such as diethyl ether or benzene. The salt normally precipitates out of solution within about one hour to 10 days and can be isolated by filtration or the solvent can be stripped off by conventional means.

Bases commonly used for formation of salts include ammonium hydroxide and alkali and alkaline earth metal hydroxides, carbonates, as well as aliphatic and primary, secondary and tertiary amines, aliphatic diamines. Bases especially useful in the preparation of addition salts include ammonium hydroxide, potassium carbonate, methylamine, diethylamine, ethylene diamine and cyclohexylamine.

The pharmaceutically acceptable salts generally have enhanced solubility characteristics compared to the compound from which they are derived, and thus are often more amenable to formulation as liquids or emulsions.

Pharmaceutical formulations can be prepared by procedures known in the art. For example, the compounds can be formulated with common excipients, diluents, or carriers, and formed into tablets, capsules, suspensions, powders, and the like. Examples of excipients, diluents, and carriers that are suitable for such formulations include the following: fillers and extenders such as starch, sugars, mannitol, and silicic derivatives; binding agents such as carboxymethyl cellulose and other cellulose derivatives, alginates, gelatin, and polyvinyl pyrrolidone; moisturizing agents such as glycerol; disintegrating agents such as calcium carbonate and sodium bicarbonate; agents for retarding dissolution such as paraffin; resorption accelerators such as quaternary ammonium compounds; surface active agents such as cetyl alcohol, glycerol monostearate; adsorptive carriers such as kaolin and bentonite; and lubricants such as talc, calcium and magnesium stearate, and solid polyethyl glycols.

The compounds can also be formulated as elixirs or solutions for convenient oral administration or as solutions appropriate for parenteral administration, for instance by intramuscular, subcutaneous or intravenous routes. Additionally, the compounds are well suited to formulation as sustained release dosage forms and the like. The formulations can be so constituted that they release the active ingredient only or preferably in a particular part of the intestinal tract, possibly over a period of time. The coatings, envelopes, and protective matrices may be made, for example, from polymeric substances or waxes.

The particular dosage of a compound of formula I required to inhibit an autoimmune disease or its symptoms, according to this invention, will depend upon the severity of the condition, the route of administration, and related factors that will be decided by the attending physician. Generally, accepted and effective daily doses will be from about 0.1 to about 1000 mg/day, and more typically from about 50 to about 200 mg/day. Such dosages will be administered to a subject in need thereof from once to about three times each day, or more often as needed to ffectively tr at or prevent the disease(s) or symptom(s).

It is usually preferred to administer a compound of formula I in the form of an acid addition salt, as is customary in the administration of pharmaceuticals bearing a basic group, such as th piperidino ring. It is pref rred to administer a compound f the invention to an aging human (e.g. a post-m nopausal female). For such purpos s th following oral dosage forms are available.

Formulations

In the formulations which follow, "Active ingredi nt" means a compound of formula I.

Formulation 1: G latin Capsules

Hard gelatin capsules are prepared using the following:

Ingredient	Quantity (mg/capsule)
Active ingredient	0.1 - 1000
Starch, NF	0 - 650
Starch flowable powder	0 - 650
Silicone fluid 350 centistokes	0 - 15

The ingredients are blended, passed through a No. 45 mesh U.S. sieve, and filled into hard gelatin capsules.
Examples of specific capsule formulations of raloxifene that have been made include those shown below:

Formulation 2: Raloxifene capsule

Ingredient	Quantity (mg/capsule)
Raloxifene	1
Starch, NF	112
Starch flowable powder	225.3
Silicone fluid 350 centistokes	1.7

Formulation 3: Raloxifene capsule

Ingredient	Quantity (mg/capsule)
Raloxifene	5
Starch, NF	108
Starch flowable powder	225.3
Silicone fluid 350 centistokes	1.7

Formulation 4: Raloxifene capsule

Ingredient	Quantity (mg/capsule)
Raloxifene	10
Starch, NF	103
Starch flowable powder	225.3
Silicone fluid 350 centistokes	1.7

Formulation 5: Raloxifene capsule

Ingr di nt	Quantity (mg/capsule)
Raloxif n	50
Starch NF	150
Starch flowable powder	397
Silicone fluid 350 centistokes	3.0

The specific formulations above may be changed in compliance with the reasonable variations provided.
A tablet formulation is prepared using the ingredients below:

Formulation 6: Tablets

Ingredient	Quantity (mg/tablet)
Active ingredient	0.1 - 1000
Cellulose, microcrystalline	0 - 650
Silicon dioxide, fumed	0 - 650
Stearate acid	0 - 15

The components are blended and compressed to form tablets.

Alternatively, tablets each containing 0.1 - 1000 mg of Active ingredient are made up as follows:

Formulation 7: Tablets

Ingredient	Quantity (mg/tablet)
Active ingredient	0.1 - 1000
Starch	45
Cellulose, microcrystalline	35
Polyvinylpyrrolidone (as 10% solution in water)	4
Sodium carboxymethyl cellulose	4.5
Magnesium stearate	0.5
Talc	1

The Active ingredient, starch, and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders which are then passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°-60° C and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 60 U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets.

Suspensions each containing 0.1 - 1000 mg of Active ingredient per 5 mL dose are made as follows:

Formulation 8: Suspensions

Ingredient	Quantity (mg/5 ml)
Active ingredient	0.1 - 1000 mg
Sodium carboxymethyl cellulose	50 mg
Syrup	1.25 mg
Benzoic acid solution	0.10 mL
Flavor	q.v.
Color	q.v.
Purified water to	5 mL

The Active ingredient is passed through a No. 45 mesh U.S. sieve and mixed with the sodium carboxymethyl cellulose and syrup to form a smooth paste. The benzoic acid solution, flavor, and color are diluted with some of the water and added, with stirring. Sufficient water is then added to produce the required volume.

ASSAYS

Assay 1

The procedure as set out in Holmdahl *et al., Clin. Exp. Immunol., 70*, 373-378 (1987) (herein incorporated by reference) is carried out. Four to thirty female mice, aged approximately 8-10 weeks, are ovariectomized. Administration of a compound of the invention is begun within three weeks after castration on the experimental group. After one week of administration of a compound of formula 1, the mice are immunized with rat type II collagen. The mice are graded for clinical severity of arthritis, as set out in Holmdahl *et al., Arthritis Rheum., 29*, 106 (1986), herein incorporated by reference. Sera are collected, and assayed for anti-type II collagen reactive antibodies. At the termination of the experiment, spleen cells are obtained from the mice for determination of T cell activity.

Activity is illustrated by a reduction in titer of anti-collagen type II antibodies determined by conventional ELISA assay. Reduction in T-cell reactivity to type II collagen presented to splenic T-cells by antigen presenting cells is evaluated by quantitation of DNA synthesis by thymidine uptake. Finally, clinical severity of disease is evaluated daily by defining first signs of erythema and swelling of one or more limbs. Clinical assessment is correlated with histologic examination.

Assay 2

Between four and thirty young adult female Sprague-Dawley rats are fed animal chow and water ad libitum. The experimental group receives a compound of formula 1, and all rats receive rat cord generally as described in Arnason *et al., Arch. Neurol., 21*, 103-108 (1969), incorporated herein by reference. The rats are graded for signs of experimental allergic encephalomyelitis (EAE). Between three and seven weeks after administration of a compound of formula 1 began, the rats are sacrificed, their spinal cords removed and examined.

Activity is illustrated by the ability of a compound to inhibit EAE.

ASSAY 3

Between five and fifty mice (MRL/lpr and NZB) are used. Reduction of anti-DNA antibodies, quantitated by ELISA, as well as changes in survival time and histologic exam of kidneys are evaluated parameters. The mice are dosed with compounds of the invention and are evaluated using the above parameters for disease progression.

ASSAY 4

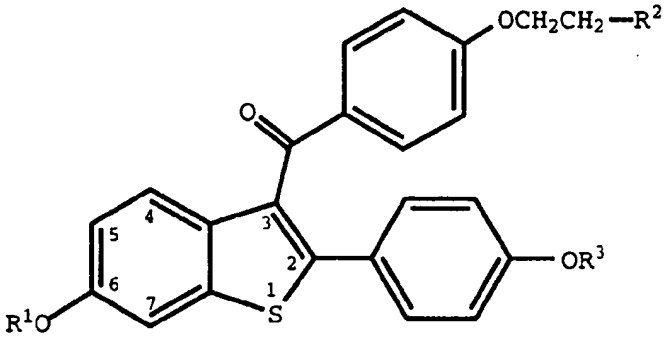
Five to fifty women are selected for the clinical study. The women are post-menopausal, i.e., have ceased

menstruating for between 6 and 12 months prior to the study's initiation, suffer from an autoimmune disease which exhibits symptoms, but otherwise are in good general health,. Because of the idiosyncratic and subjective nature of these disorders, the study has a placebo control group, i.e., the women are divided into two groups, one of which receives a compound of formula 1 as the active agent and the other receives a placebo. Women in the test group receive between 50-200 mg of the drug per day by the oral route. They continue this therapy for 3-12 months. Accurate records are kept as to the number and severity of the symptoms in both groups and at the end of the study these results are compared. The results are compared both between members of each group and also the results for each patient are compared to the symptoms reported by each patient before the study began.

Utility of the compounds of formula I is illustrated by the positive impact they have in at least one of the assays described above.

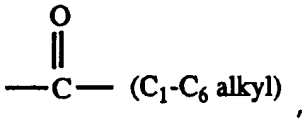
Claims

1. The use of a compound having the formula

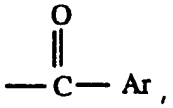


(I)

wherein R^1 and R^3 are independently hydrogen, $-CH_3$,



or



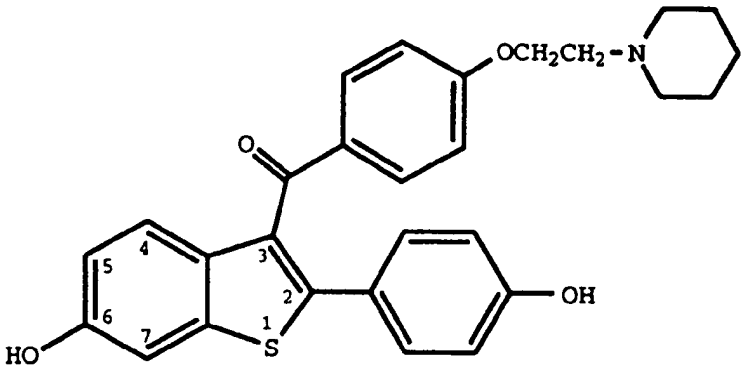
wherein Ar is optionally substituted phenyl;

R^2 is selected from the group consisting of pyrrolidino and piperidino; or a pharmaceutically acceptable salt or solvate thereof, in the preparation of a medicament useful for inhibiting an autoimmune disease.

2. The use of Claim 1 wherein said compound is the hydrochloride salt thereof.

3. The use of Claim 1 wherein said medicament is prophylactic.

4. The us of Claim 1 wherein said comp und is



or its hydrochloride salt.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 94 30 9465

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X Y	US-A-5 075 321 (SCHREIBER) * column 2; claims 29-53 * ---	1-4 1-4	A61K31/445 A61K31/40
Y	ACTA ONCOLOGICA, vol. 31, no.2, 1992 pages 143-146, L. KANGAS 'Agonistic and antagonistic effects in different target organs' * page 145; table 2 * ---	1-4	
A	DATABASE FILE 187, FDC REPORTS Dialog Information Services 1987-1993, Accession No. 00089403, *on-line abstract* & THE PINK SHEET, vol. 55, no.16, 19 April 1993 'Lilly's raloxifene entering phase III for osteoporosis' -----	1-4	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

A61K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	6 April 1995	Foerster, W

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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E : earlier patent document, but published on, or after the filing date
D : document cited in the application
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